Supplementary Material

S100A10 promotes HCC development and progression via transfer in extracellular vesicles and regulating their protein cargos

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Supplementary Materials and Methods

Human Samples

Plasma samples were randomly collected from HCC patients without treatment and healthy subjects without liver disease background. Human HCCs and their paired non-tumorous liver (NT) tissues were collected during surgical resection at Queen Mary Hospital, Hong Kong, with informed consent from patients. Procedure approval was obtained from the Institutional Review Board of The University of Hong Kong. All experiments involving human samples were handled in accordance with relevant ethical regulations.

Cell lines and culture conditions

HCC cell line PLC/PRF/5 (CRL-8024) was obtained from American Type Culture Collection (ATCC). HCC cell line Huh7 (JCRB0403) was obtained from JCRB Cell Bank. MHCC97L and MHCC97H were gifts from Liver Cancer Institute, Fudan University. MIHA was kindly provided by Dr. J.R. Chowdhury, Albert Einstein College of Medicine, New York. MHCC97L cells were cultured in Dulbecco's modified Eagle minimal high glucose essential medium (DMEM-HG) supplemented with 1mM NaPy. PLC/PRF/5, Huh7, MIHA and HEK293FT cells

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were cultured in DMEM-HG media. All cell culture media mentioned above were further supplemented with 10% fetal bovine serum (FBS), 1% penicillin and 1% streptomycin unless otherwise specified. Cell line cultures were maintained in 37ºC and 5% CO2 incubator. Authentication of HCC cell lines used in this study was performed by short tandem repeat (STR) DNA Profiling in March 2018 and no cellular cross-contamination was detected. STR results for MHCC97L is provided in Supplementary figure 2. Cell cultures were tested negative for Mycoplasma contamination. "Xenome", utilizing our RNA-seq data, estimated a negligible 0.04% to 0.42% (n=3) "mouse-likely" reads (probably an artifact instead of real contamination) for MHCC97L, which was comparable to the 0.15% to 0.40% for clinical human NTL and HCC samples (n=6), thus indicating our MHCC97L cells do not contain cells of murine origin (1). Furthermore, MHCC97L used in this study contains HBV integrated in TERT locus of the genome (2).

Lentiviral-based S100A10 overexpression cells and CRISPR/Cas9 mediated S100A10-KO cells

S100A10 lentiviral-based expression constructs using pCDH-EF1-MCS-IRES-Puro vectors (System Biosciences) were prepared by standard molecular cloning techniques. Lenti-Guide-Puro based lentiviral single guide RNA (sgRNA) expression vector (Provided by Dr. Zheng Feng, MIT) carrying specific S100A10 targeting sequence (sgS100A10) or GFP targeting control sequence (sgGFP) transfected into 293FT packaging cells to produce viral containing supernatant for subsequent HCC cells viral transduction of Cas9 expressing MHCC-97H cells transduced by lenti-Cas9-Blast system (Dr. Zhang Feng, MIT). To establish S100A10 KD, two short hairpin RNAs (shRNA) specifically targeting S100A10 (shS100A10-1, and shS100A10-3) were cloned into the PLL3.7 lentiviral vector (Addgene). Stably transduced cells were

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selected by puromycin (Sigma-Aldrich). Targeted sequences for gene of interest are described in Table S3.

Focus formation assay

Focus formation assay was used to assess anchorage-dependent growth. In brief, 1,000 cells were seeded in each well of six-well plate for approximately 2-3 weeks. Surviving colonies were stained and counted using crystal violet (Sigma-Aldrich).

Sphere formation assay

A total of 1,000 cells were cultured in 0.25% methyl cellulose (Sigma-Aldrich) supplemented DMEM/F12 medium (Life Technologies) with 20ng/mL EGF (Life Technologies), 10ng/mL basic FGF (Life Technologies), B27 (1:50, GIBCO), and 4μg/mL insulin (BIOIND, Kibbutz Beit Haemek, Israel) in 24-well plates, which were coated with poly HEMA (Sigma-Aldrich). The cells were replenished with 30 μL supplementary medium every other day.

Cell motility assays

Transwell migration and invasion assays were performed to evaluate cell motility (Corning, #353097 for migration, #354480 for invasion). Approximately 1×10^5 cells were seeded in the medium without FBS on transwell upper chambers, and the lower chamber was supplied with medium with 10% FBS, with the indicated conditional medium, or EVs. The cells that migrated and invaded to the lower membrane surface were stained using crystal violet and then counted under microscopy.

Animal studies

All animal experiments were conducted and approved by the University of Hong Kong Committee on the Use of Live Animals in Teaching and Research (CULATR). The frequency of CSCs with tumor initiation capabilities was evaluated using limiting dilution assay. The BALB/c nude mouse xenograft model was used to evaluate tumorigenicity and chemoresistant ability in vivo. Nude mice were intrasplenically injected with HCC cells to assess the liver metastatic capacity *in vivo*. After surgery, mice recovered from anesthesia in a cage under a heater. Analgesia was provided to the nude mice during the first postoperative week. Tail vein injection of luciferase-labelled MHCC-97L cells in NOD SCID mice was used to assess lung metastatic ability. After mice were killed, the tumor tissues, livers, spleens, and lungs were excised and fixed in 4% paraformaldehyde overnight. Thereafter, fixed tissues were embedded in paraffin for further studies. For chemoresistance in xenograft model, subcutaneous xenografts in nude mice were established with PLC/PRF/5 or MHCC97L cells. Treatment was started once the size of the xenograft reached ~5mm in diameter. The mice were randomly assigned into different groups, each consisting of at least 5 mice. Sorafenib at 10 mg/kg was administered daily through oral gavage. Mouse IgG antibody or anti-S100A10 antibody was administered at 10 μg once every 3 days by intraperitoneal injection.

Chemotherapy-induced cytotoxicity and apoptotic assay. Sorafenib, cisplatin or 5-FU induced cytotoxicity was determined by XTT Cell Proliferation Assay (Roche Diagnostics) according to the manufacturer's instructions. The apoptotic assay was determined by flow cytometry using annexin-V staining. After treating with Sorafenib, cisplatin or 5-FU for 48 h, the cells were collected and double stained with FITC-conjugated Annexin-V and PI provided in the BD apoptosis detection kit (BD Biosciences).

Isolation of EVs from cell culture medium and plasma of HCC patients

For EVs isolation from cell culture supernatants, HCC cells were cultured in medium with 10% EV-depleted FBS, which was prepared by 100,000× g centrifugation overnight (⩾12h) at 4 °C (Himac, CP100NX Ultracentrifuges). EVs were purified by differential centrifugation after the cell culture supernatant were collected. Briefly, cell culture supernatants were centrifuged at 2000×g for 15 min to remove cell debris and dead cells. Then the supernatant was centrifugated at 20 000×g for 30 min at 4°C to remove microvesicles. Then the supernatant was first passed through 0.22 μm filter followed by ultracentrifugation at 100,000×g for 2 h at 4 °C to collect EVs. The EVs were then washed with PBS and collected by ultracentrifugation at 100,000×g for another 2 h at 4°C. HCC patients' or healthy donors' plasma derived EVs were purified by differential centrifugation. The plasma was first centrifuged at 4000g for 15 min to obtain cell-free plasma. Then, 500μL of the obtained plasma was toped-up to 1 mL and then centrifuged at 20,000g for 1 hour (Himac). The collected supernatants were then centrifuged at 100,000g for 2 h at 4 °C (Himac) to pellet the EVs. The EVs were then washed with PBS and collected by ultracentrifugation at 100,000×g for another 2 h at 4°C.

EV characterization

The morphology and integrity of EVs is observed by electronic microscope. In brief, EVs suspended in PBS were dropped on formvar carbon-coated nickel grids and stained with 2% uranyl acetate. The EVs were then visualized by Philips CM100 transmission electron microscope (FEI Company). Target proteins present on EVs were determined by immunogold staining followed by visualizing by transmission electron microscope. Protein of isolated EVs was examined by western blotting by EV specific markers CD63 (Abcam, 134045), CD81 (Abcam, #79559), CD9 (Abcam, #92726), HSP70 (Abcam, #181606), Alix (Santa Cruz, #53540), TSG101 (BD Biosciences, #612696) and EVs negative markers GM130 (Abcam,

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#52649) and p62 (Abcam, 140651). The size distribution of EVs and particle concentration was measured by ZetaView BASIC NTA PMX-120 (Particles Metrix GmbH).

EV education mouse model

To investigate the role EVs in tumor liver metastasis, 6-week-old BALB/c nude male mice were intrasplenic injected with HCC cells. After the implantation of tumor cells, 10μg EVs or PBS was intravenously injected every 4 days for 1 month. Autopsies were performed after 8-10 weeks and the presence of metastases was examined macroscopically. For lung metastasis, NOD SCID mice were injected intravenously with luciferase-labelled HCC cells and 10μg EVs or PBS as control. Then the EVs or PBS was educated to mice every 4 days for 1 month. Lung metastasis was monitored using bioluminescence imaging at around 8-10 weeks.

Labeling of EVs for Uptake Analysis

EVs were fluorescently labeled with PKH26 Membrane Dye Labeling Kit (Sigma Aldrich, #PKH26GL) according to manufacturer's protocol. Labeled EVs were washed with PBS and collected by ultracentrifugation as described above. To assess tissue distribution of EVs, NOD SCID mice were injected intravenously with 15 μg of PKH26-labeled EVs. Each mouse was anesthetized and perfused to collect lung, liver, spleen, heart and kidney. Tissue sections from different organs were stained with DAPI and examined under LSM900 confocal microscopy (Carl Zeiss). Three random fields of each section were captured and three sections per organ were examined.

Pulmonary leakiness assay

Male 6-week-old NOD SCID mice were injected intravenously with 15 μg of EVs or PBS as control. They were intravenously injected with Texas Red lysine-fixable dextran (Invitrogen,

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#D1864, 70,000MW) at 100 mg/kg post 24 hr EV injection. Mice were intravenously injected with Alexa Fluor 488 concanavalin A (Invitrogen, #C11252) at 10 mg/kg after 3 hr. They were anesthetized 10 min later and lung tissues were excised. Tissues were cryosectioned at 12 μm thickness. Tissue sections were stained with DAPI (Invitrogen, #D1306) and examined under LSM900 confocal microscopy (Carl Zeiss) for vascular leakage. Three random fields of each section were captured and 3 sections per lung were examined.

Treatment of HCC cells before functional assays

HCC cells were seeded in 6-well plate and subjected to 2μg/mL EVs in DMEM (with 10% FBS) treatment for 48 hours one day after seeding. Then the cells were used for functional studies or western blotting. For treatment with neutralizing antibody (NA) or MMP2 inhibitor, EVs were incubated with PBS (1:10 volume/volume), IgG antibody (1:10 volume/volume) (Santa Cruz, sc-2025), anti-S100A10 NA (1:10 volume/volume) (Santa Cruz, sc-81153), or MMP2 inhibitor (OA-Hy, Calbiochem 444244, 100nM) before they were applied in vitro or in vivo assays[1]. For S100A10 NA or ITGAV treatment before EVs isolation, 20ug S100A10 NA or IgG was pre-incubated with 1,000 million cells, or 100ug GRGDSP peptides (RGD, Sigma-Aldrich, SCP0157) or the control GRADSP peptides (RAD, Sigma-Aldrich, SCP0156) were pre-incubate with 1,000 million cells and then seed in medium with 10% EVs free FBS. Then the EVs were collected after 48 hours.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using the TRIZOL Reagent (Life Technologies). cDNA was synthesized by reverse transcription (Roche). SYRB Green PCR Master Mix (Applied Biosystems) and Applied Biosystems Quant Studio 5 Real-time PCR System were used for

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qRT-PCR analysis. All qRT-PCR reactions were tested in triplicates. Primers used in this study are listed in Supplementary Table 1.

Gene copy number variation assay

TaqMan probe-based gene copy number assay was used to quantify the copy number variation (CNV) of S100A10. Copy number of S100A10 targeting intron 1 (Hs06508510_cn, Cat. No. 4400292, Life Technologies, CA, USA) was measured. TaqMan™ Copy Number Reference Assay, human, RNase P (Life Technologies, #4403326) was used as internal reference control. The CNV of paired HCC and their corresponding non-tumorous liver (NT) tissues were normalized to normal liver tissue.

Western blotting analysis

Quantified protein lysates were resolved on SDS-PAGE, transferred onto a polyvinylidenedifluoride (PVDF) membrane (Bio-Rad), and then blocked with 5% non-fat milk in Tris-buffered saline-Tween 20 (TBST) for 1 h at room temperature. The blocked membrane was then incubated with primary antibody diluted in 5% bovine serum albumin in TBST at 4 °C overnight. Band intensities of western blot were analyzed using ImageJ. Antibodies used in this study are listed in Supplementary Table 2.

Gelatin zymography

50 μg un-denatured EVs or 20 ng of activated recombinant MMP-2 (Abcam, #81550) was loaded with Laemmli Sample Buffer (Bio-Rad, #1610747) onto 10% gelatin zymogram protein gels (Thermo, #ZY00102BOX) and run at 100V for 1.5 h in Tris-Glycine SDS Running Buffer (Thermo, #LC2675). Gels were then incubated with 1× renaturing buffer (Thermo, #LC2670) for 30 min at room temperature and then incubated with 1× developing buffer (Thermo,

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#LC2671) overnight at 37 °C. Developed gels were then gently washed with H_20 and stained with Coomassie Blue dye.

Statistical analysis

The SPSS version 17.0 (SPSS, Inc., Chicago, IL) was used for data analysis. The mRNA level of S100A10 in paired tumor and adjacent nontumor tissues was compared with a paired Student t test. Patients' survival rates were analyzed using Kaplan-Meier plots and log-rank tests. The correlations between different clinicopathological parameters were evaluated using Fisher exact test. The frequency of CSCs with tumor initiation capabilities were calculated by limiting dilution assay in the ELDA software[2]. Data are presented as the mean ± SD of three independent experiments. Results were considered statistically significant for P values <0.05.

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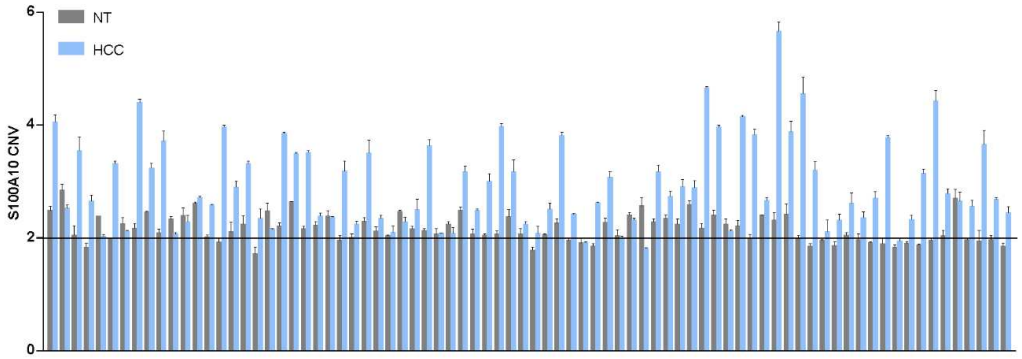
Supplementary Figures

A

Association of S100A10 expression with clinicopathologic features in TCGA

Features	Total	S100A10 expression*		*P*-value
		Low	High	
Tumor stage (AJCC)	347			0.714
Stage Ⅰ - Ⅱ	257 (74.1%)	125 (73.1%)	132 (75.0%)	
Stage Ⅲ-Ⅳ	90 (25.9%)	46 (26.9%)	44 (25.0%)	
Virus infection	352			**<0.001**
Absent	199 (56.5%)	117 (66.5%)	82 (46.6%)	
Present	153 (43.5%)	59 (33.5%)	94 (53.4%)	
Alcohol uptake	352			0.258
Yes	117 (33.2%)	53 (30.1%)	64 (36.4%)	
No	235 (66.8%)	123 (69.9%)	112 (63.6%)	

B



Supplementary figure 1. Clinical significance of S100A10. A. Correlation between S100A10 expression and clinicopathological features in TCGA database. Fisher exact test. **B.** Copy number variation (CNV) of S100A10 detected by TaqMan Copy Number Assay in paired HCC and corresponding nontumorous liver tissues (NT).

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DNA Marker	MHCC97L[4]	MHCC97L (L-171218744P)
AMEL	X, Y	X, Y
CSF1PO	11, 13	11, 13
D13S317	8	8
D16S539	12	12
D5S818	12,13	12,13
D7S820	10	10
TH01	9	9
TPOX	8	8
vWA	14	14
D18S51	--	13, 22
D21S11	--	31.2
D3S1358	--	15, 16
D8S1179	--	12, 13
FGA	--	21, 24
Penta D	--	8, 9
Penta E	--	11, 17
Number of shared alleles		12
Total number of alleles in the reference profile		12
Percent match		100%

Supplementary figure 2. Short tandem repeat (STR) DNA profiling of MHCC97L, authenticating no contamination.

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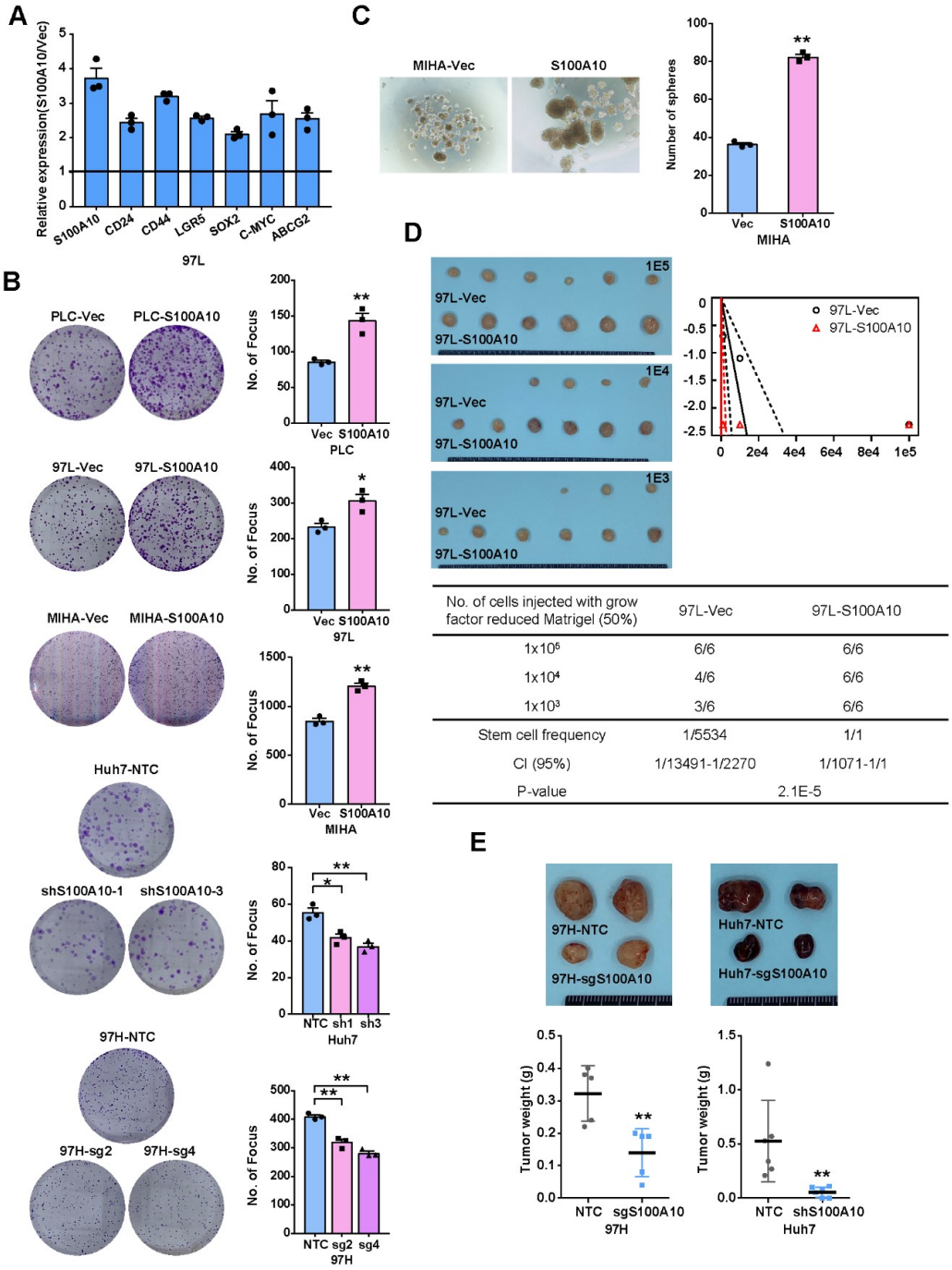

No. of cells injected with grow factor reduced Matrigel (50%)	97L-Vec	97L-S100A10
$1x10^5$	6/6	6/6
$1x10^4$	4/6	6/6
$1x10^3$	3/6	6/6
Stem cell frequency	1/5534	1/1
CI (95%)	1/13491-1/2270	1/1071-1/1
P-value	2.1E-5	

Supplementary figure 3. S100A10 promotes the tumorigenicity of HCC. A. The relative mRNA expression of HCC stemness-related markers of CD24, CD44, LGR5, SOX2, c-MYC and ABCG2 in 97L-S100A10-OE as compared with Vec control using qPT-PCR. B. Focus

formation assays of S100A10/Vec, shS100A10s/NTC and sgS100A10s/NTC in PLC, 97L, MIHA, Huh7 and 97H cells, respectively. C. Sphere formation ability induced by MIHA-S100A10. D. Limiting dilution assays of 97L-Vec/S100A10. The tumor-initiating frequencies are summarized in chart and table form. E. Subcutaneous tumors formed by 97H-sgS100A10#4 and Huh7-shS100A10#3 were much smaller than their NTC counterparts; Mean ± SD of 5-6 mice.

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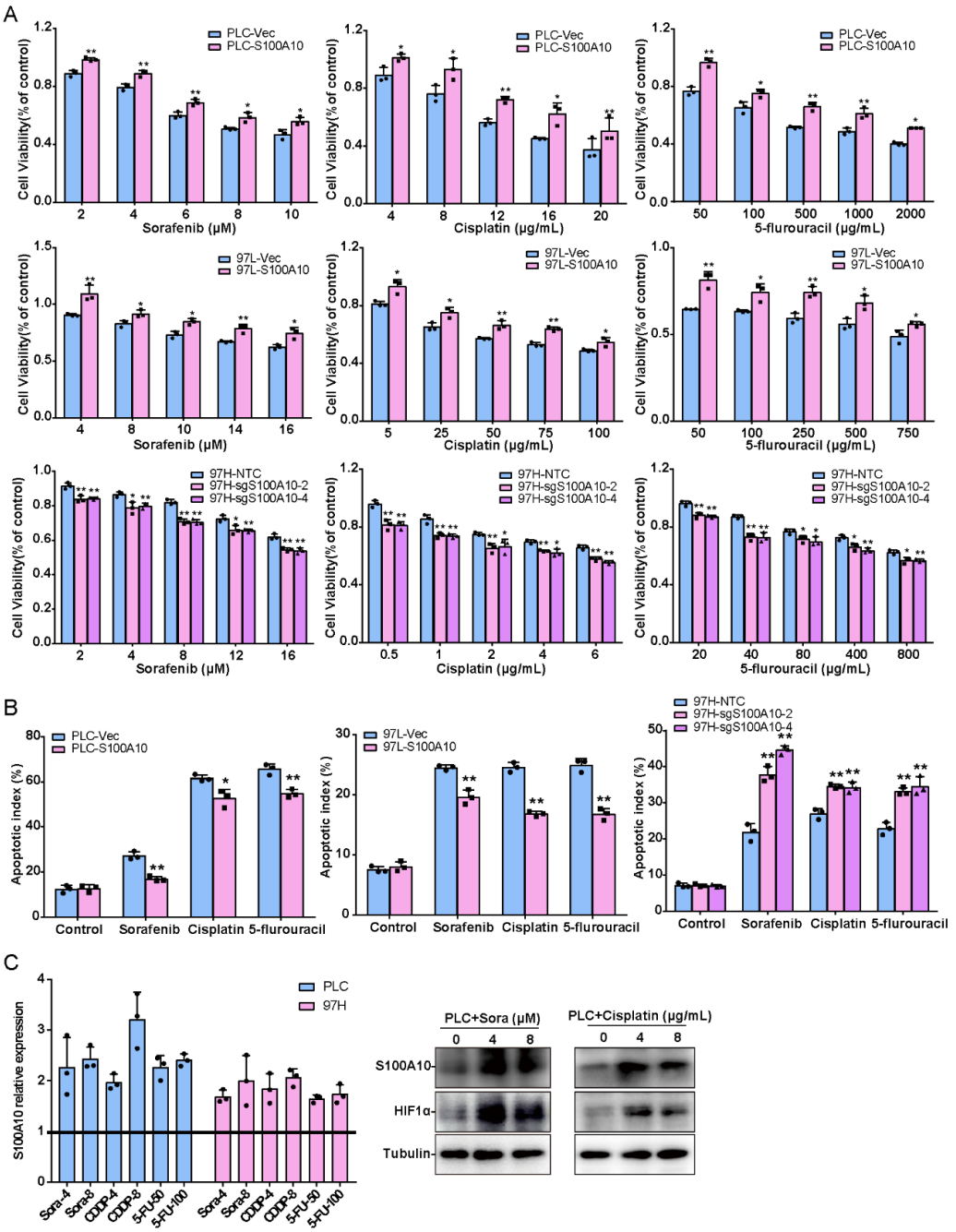

Supplementary figure 4. S100A10 enhances chemoresistance of HCC. A. XTT assay showing chemoresistance of PLC-Vec/S100A10, 97L-Vec/S100A10 and 97H-NTC/sgS100A10s to sorafenib, cisplatin and 5-FU. B. Apoptosis analysis using annexin-V staining and flow cytometry on PLC-Vec/S100A10, 97L-Vec/S100A10 and 97H-

NTC/sgS100A10s treated with sorafenib, cisplatin and 5-FU, respectively. C. Relative S100A10 expression detected by qRT-PCR under treatment as indicated (left panel): Sora-4, Sora-8: sorafenib at 4 or 8 μM; CDDP-4, CDDP-8: cisplatin at 4 or 8 μg/mL; 5-FU-50, 5-FU-100: 5-FU at 50 or 100 μg/mL. S100A10 and HIF-1α expression detected by western blot (right panel). Tubulin was used as a loading control.

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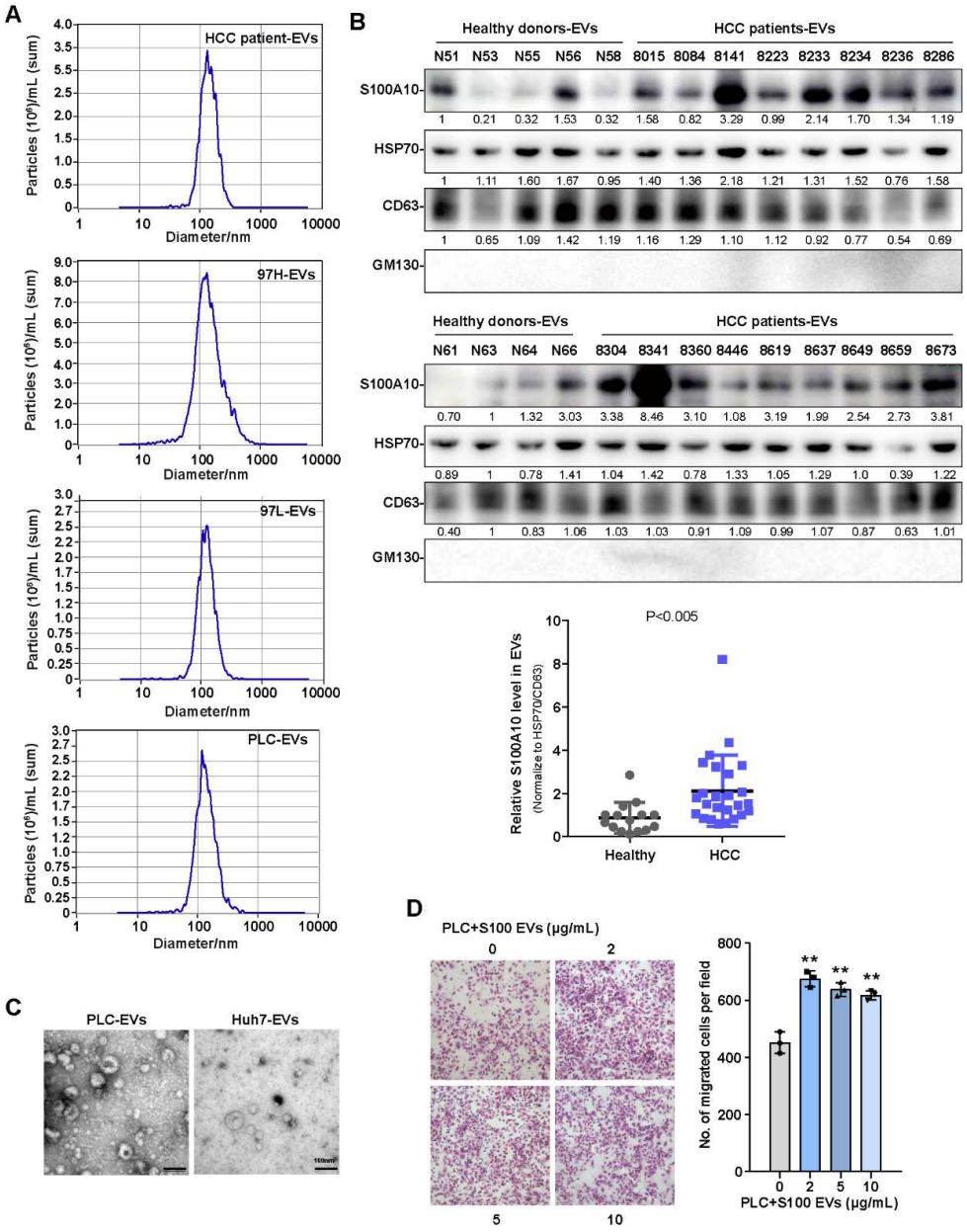
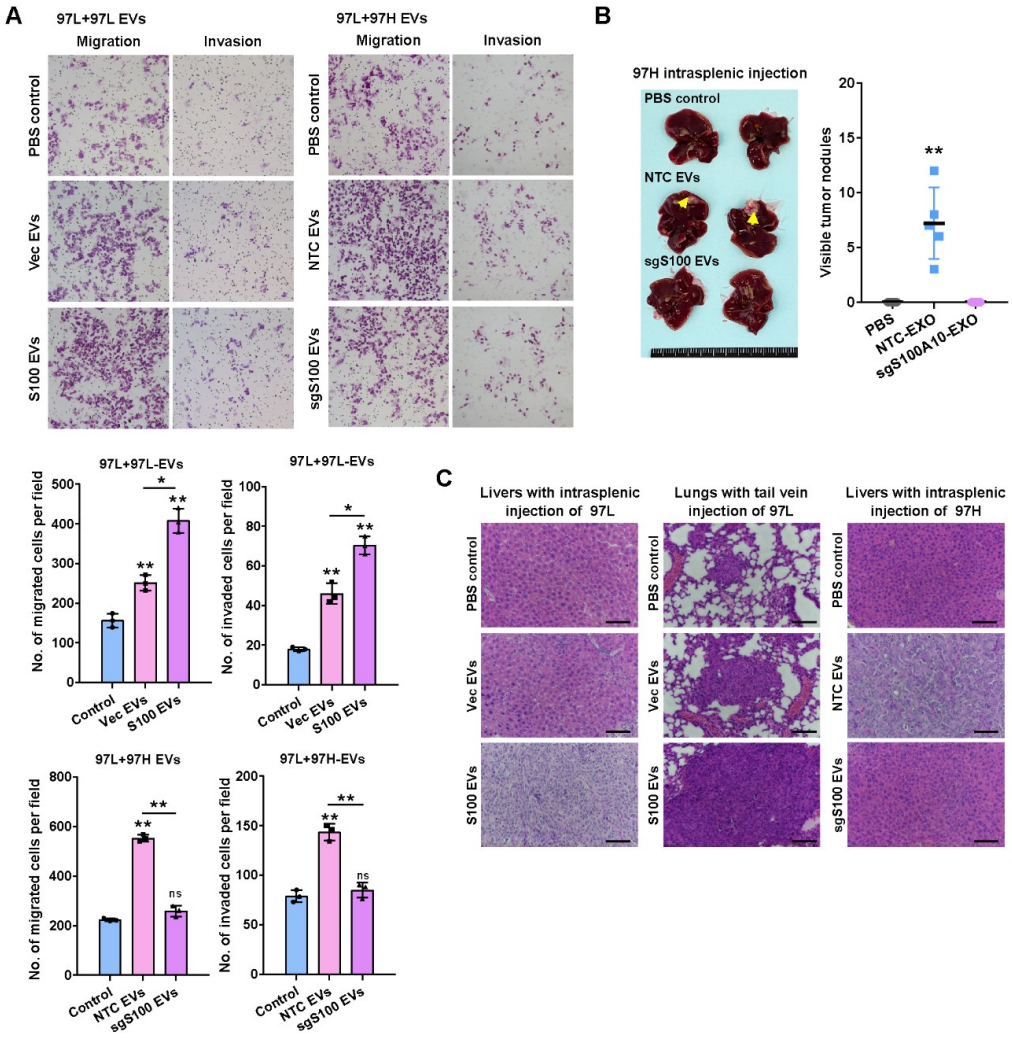


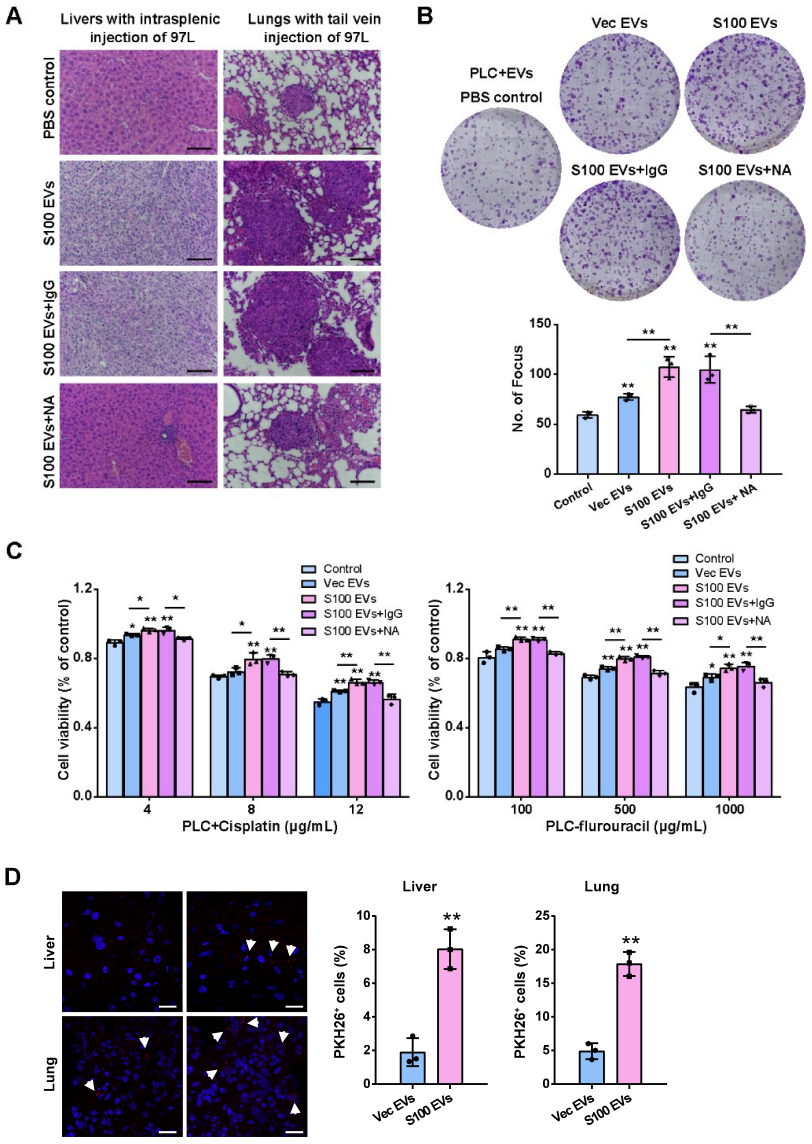
Supplementary figure 5. S100A10 is present in HCC-derived EVs and promotes HCC motility. A. Size distribution of EVs from HCC patient's plasma, 97H, 97L, and PLC cells, as measured by ZetaView Particle Tracking Analyzer. B. Western blot showing detection of S100A10 and EVs markers including CD63, HSP70, and Golgi marker GM130 in EVs from HCC patients' plasma. Each lane represents either a healthy donor or a HCC patient. The levels of S100A10 in EVs from all clinical samples were quantified and normalized to the average of

HSP70 and CD63 (lower panel). C. Representative electron micrograph showing the morphology of EVs from PLC and Huh7 cells. Scale bar = 100 nm. D. Cell migration assay of PLC cells treated with EVs from S100A10-OE cells (S100 EVs) at indicated concentrations.



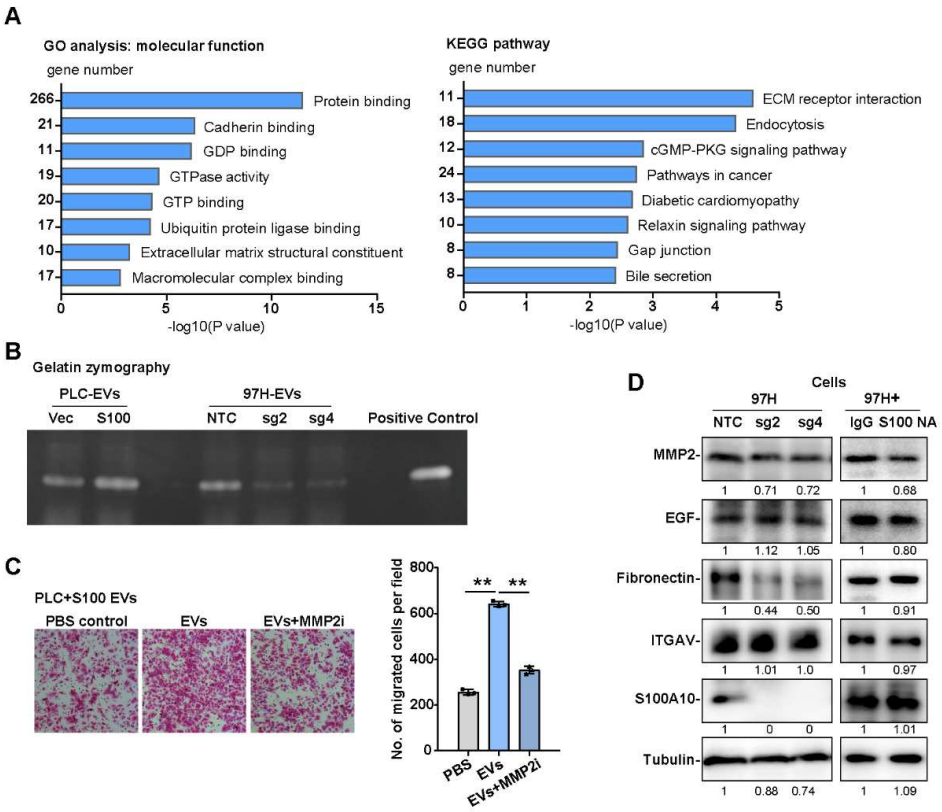
Supplementary figure 6. S100A10 EVs promote HCC motility and metastasis. A. Cell migration and invasion assays of 97L cells treated with EVs from the corresponding vector control cells (Vec EVs) or S100A10-OE cells (S100 EVs), or with EVs from 97H-NTC (NTC EVs) or -sgS100A10#4 (sgS100 EVs) cells. B. Livers in the intrasplenic injection model of

97H cells in nude mice educated with PBS or indicated EVs. C. Representative histology with H&E stain of the corresponding liver or lung sections as indicated. Scale bar = 100 μm.



Supplementary figure 7. S100A10 in EVs plays a key role in inducing HCC stemness features. A. Representative histology with H&E stain of the corresponding liver or lung sections as indicated. Scale bar = 100 μm. Focus formation (B) and chemoresistance (C) were assessed on PLC cells treated with PBS, EVs from vector control cells (Vec EVs), or from

S100A10-OE cells (S100 EVs). The EVs were preincubated and treated together with S100A10 NA or IgG control antibody. D. Representative image of the distribution of EVs derived from 97L-Vec or S100A10-OE in livers and lungs of mice. The mice were sacrificed 24 h after intravenously injected with EVs labeled with PKH26. Tissues were subjected to frozen sections and examined under confocal microscopy. $PKH26^{+}$ EVs as seen in red color are indicated by the arrowhead. DAPI (blue) was used for nuclei counterstaining. Quantification of the percentage of $PKH26^{+}$ cells in three random fields of three tissue sections per organ is shown. Scale bar = 20 μm.



Supplementary figure 8. S100A10 alters the protein content of EVs. A. Mass spectrometry (MS) was performed to compare the different proteins in 97L-Vec-EVs and 97L-S100A10-EVs. Gene ontology (GO) analysis and KEGG analysis were used to identify the molecular functions and signaling pathways enriched in S100A10 upregulated proteins in EVs. B. EVs

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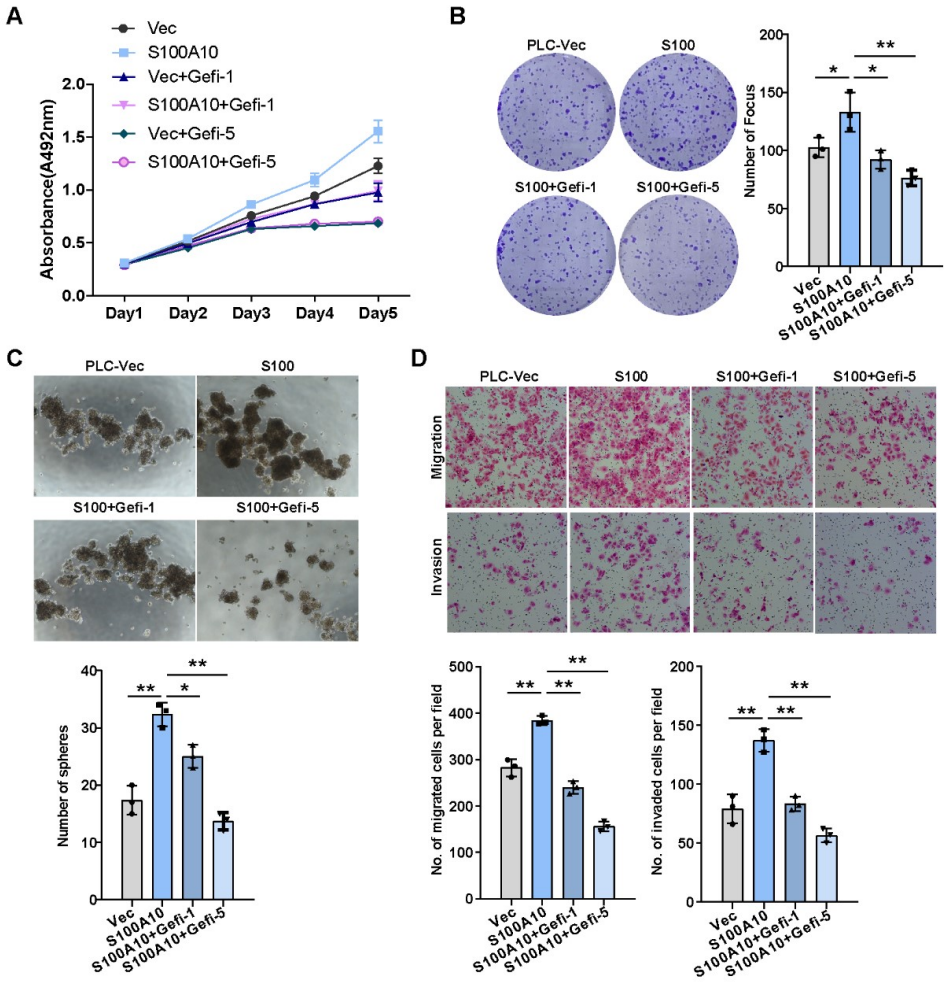
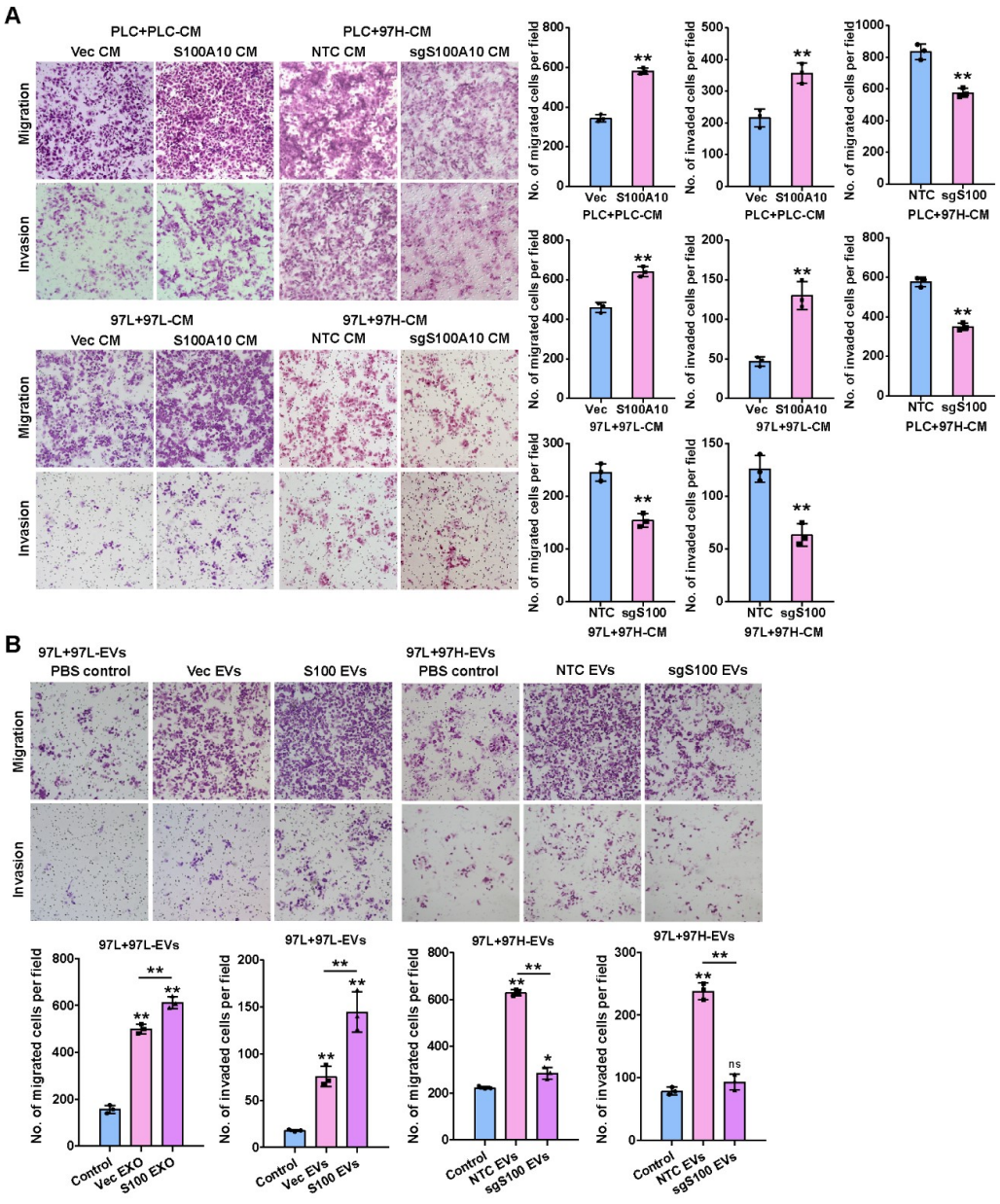
derived from PLC-Vec/S100A10 or 97H-NTC/sgS100A10s were subject to zymography assay to measure the MMP-2 activity. 20 ng of recombinant MMP-2 served as positive controls. C. Migration assay of PLC cells treated with S100 EVs with or without MMP2 inhibitor (MMP2i). D. Western blot showing the expression levels of S100A10, MMP2, EGF, fibronectin and ITGAV in 97H-NTC/sgS100A10s or 97H cells treated with S100A10 NA or IgG.



Supplementary figure 9. EGFR inhibitor Gefitinib suppresses the oncogenic function of S100A10. A. XTT assay on PLC-Vec/S100A10 treated with different concentrations of Gefitinib. Focus formation (B), sphere formation (C), migration and invasion assays (D) of PLC-Vec/S100A10 treated with Gefitinib as indicated. Gefi-1, Gefi-5: Gefitinib at 1 or 5 μM.



Supplementary figure 10. EV-S100A10 promotes chemotaxis of HCC cells. A. PLC and 97L migration and invasion assays using conditional medium (CM) derived from corresponding Vec (Vec CM), S100A10-OE (S100A10 CM), or 97H-NTC (NTC CM)/-sgS100A10#4 (sgS100A10 CM) in the lower compartment of transwell. B. Migration and invasion assays on 97L cells. EVs derived from 97L-Vec (Vec EVs)/S100A10 (S100 EVs), or

97H-NTC (NTC EVs)/-sgS100A10#4 (sgS100 EVs) were added to the lower compartment of transwell chambers as indicated.

Supplementary References

1 Luo F, Tran AP, Xin L, Sanapala C, Lang BT, Silver J*, et al.* Modulation of proteoglycan receptor PTPσ enhances MMP-2 activity to promote recovery from multiple sclerosis. Nature communications 2018;**9**:4126.

2 Hu Y, Smyth GK. ELDA: extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. Journal of immunological methods 2009;**347**:70-8.

Supplementary Tables

Supplementary Table 1. List of primers used in this study.

Gene	Primer	Sequence
For Real-time PCR		
S100A10	Forward	TCGCTGGGGATAAAGGCTAC
	Reverse	AAGAAGCTCTGGAAGCCCAC
CD24	Forward	GCTCCTACCCACGCAGATTT
	Reverse	GAGACCACGAAGAGACTGGC
CD44	Forward	TGCCGCTTTGCAGGTGTAT
	Reverse	GGCCTCCGTCCGAGAGA
LGR5	Forward	CCCGAATCCCCTGCCCAGTCT
	Reverse	TCATCCAGCCACAGGTGCCTA
SOX2	Forward	AAATGGGAGGGGTGCAAAAGAGGAG
	Reverse	CAGCTGTCATTTGCTGTGGGTGATG
C-MYC	Forward	CGTCCTCGGATTCTCTGCTC
	Reverse	GCTGGTGCATTTTCGGTTGT
ABCG2	Forward	TCATCAGCCTCGATATTCCATCT
	Reverse	GGCCCGTGGAACATAAGTCTT
GAPDH	Forward	GGAGCGAGATCCCTCCAAAAT
	Reverse	GGCTGTTGTCATACTTCTCATGG

Supplementary Table 2. List of antibodies used in this study.

Antibody	Application	Source[a]	Cat No.
Anti-human S100A10	WB, IP, Immunogold	Sigma	HPA003340
Anti-human CD63	WB	Abcam	ab134045
Anti-human CD63	Immunogold	Abcam	ab271286
Anti-human CD9	WB	Abcam	ab92726
Anti-human CD81	WB	Abcam	ab79559
Anti-human TSG101	WB	BD	612696
Anti-human Alix	WB	Santa Cruz	sc-53540
Anti-human GM130	WB	Abcam	ab52649
Anti-human p62	WB	Abcam	ab140651
Anti-human HSP70	WB	Abcam	ab181606
Anti-human S100A10	Neutralization	Santa Cruz	sc-81153
Normal mouse IgG	Neutralization	Santa Cruz	sc-2025
Anti-human EGFR	Neutralization, WB	Sigma	05-101
Anti-human EGFR (phospho Y1068)	WB	Abcam	ab40815
Anti-human EGF	WB, immunogold	Santa Cruz	sc-374255
Anti-human ITGAV	WB, immunogold	Abcam	ab179475
Anti-human MMP2	WB, immunogold	Abcam	ab97779
Anti-human E-cadherin	WB	Cell signaling	3195
Anti-human Fibronectin	WB, immunogold	Abcam	ab2413
Anti-human N-cadherin	WB	Cell signaling	13116
Anti-human Vimentin	WB	Cell signaling	5741
Anti-human p44/42 MAPK (ERK1/2)	WB	Cell signaling	4695
Anti-human Phospho-p44/42 MAPK (ERK1/2)	WB	Cell signaling	4370
Anti-human Akt	WB	Cell signaling	9272
Anti-human phospho-Akt (Ser473)	WB	Cell signaling	9271

[a]Cell Signaling Technology, Danvers, MA, USA

Abcam, Cambridge, MA, USA

BD Biosciences, CA, USA

Santa Cruz Biotechnology, CA, USA

Sigma-Aldrich, St. Louis, MO, USA

Supplementary Table 3. List of target sequences for knockdown and knockout of genes.

sh/sgRNA clones	Gene name	Target sequence
shS100A10#1	S100A10	CCATGATGTTTACATTTCACA
shS100A10#3	S100A10	CCATTGCATGCAATGACTATT
sgS100A10#2	S100A10	GGAGGACCTGAGAGTACTCA
sgS100A10#4	S100A10	GTAGTACACATGAAGCAGAA
shITGAV	ITGAV	CACTCCAAGAACATGACTATT